On presence of PCR inhibitors in chiropteran fecal samples used for cataloguing virus isolates

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Problem Statement

Polyphenolic compounds are known to the PCR testing industry to inhibit polymerase. This precis seeks to answer the question, “Are PCR inhibitors present in chiropteran fecal samples used for cataloguing virus isolates?”

promega.es

Table 1. Known PCR Inhibitors.

Inhibitor	Source of Inhibitor	Reference
bile salts	feces	9*
complex polysaccharides	feces, plant material	10*
collagen	tissues	11*
heme	blood	12*
humic acid	soil, plant material	13*,14
melanin and eumelanin	hair, skin	15*,16
myoglobin	muscle tissue	17*
polysaccharides	plants	18*
proteinases	milk	19*
calcium ions	milk, bone	20*
urea	urine	21*
hemoglobin, lactoferrin	blood	22*
immunoglobin G (IgG)	blood	23*
indigo dye	denim	24

*Reviewed in reference 25.

AA sfamjournals.onlinelibrary.wile

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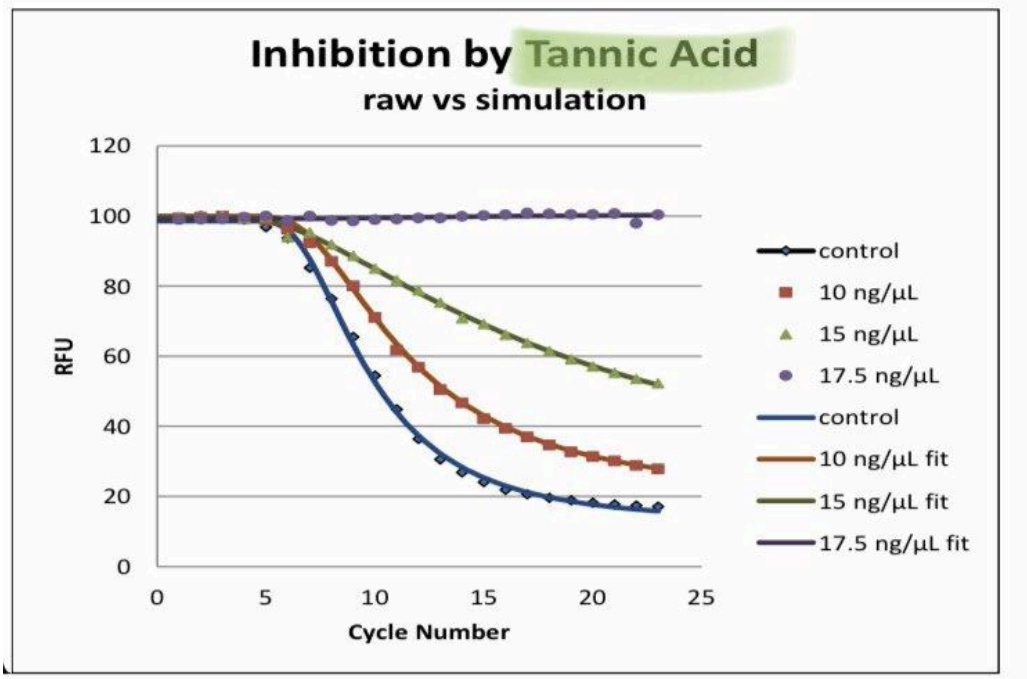
Berries	Phenols, Polysaccharides	Seeram *et al.* (2006); Wei *et al.* (2008)
Plants	Pectin, Polyphenols, Polysaccharides, Xylan	Demeke and Adams (1992); Henson and French (1993); John (1992); Sipahioglu *et al.* (2006); Su and Gibor (1988); Wan and Wilkins (1994); Wei *et al.* (2008); Wilkins and Smart 1996

The result of an inhibited polymerase in a PCR assay is a false-negative result for the virus intended for sequencing.



Demonstrating false-negative result of tannic acid, a frequently encountered polyphenolic compound, in a PCR assay. (McCord 2015)

Ecological background

Broadly:
During long-term co-evolution with insects, plants developed a series of physical, chemical, and developmental defense mechanisms to prevent insect feeding, among which chemical defense systems involving secondary metabolites are the most important (Gong and Zhang 2014). To respond plant defense, insects developed multiple resistance mechanisms against plant secondary metabolites (Ivie et al. 1983, Zhu-Salzman and Zeng 2010). From the perspective of plants, strategies to prevent phytophagous insects from feeding on plants are beneficial, including antifeeding, repelling, and poisoning strategies as well as the inhibition of insect growth and development (Kelly and Curry 1991, Panzuto et al. 2002). From the perspective of phytophagous insects, ingested plant secondary metabolites can be tolerated or detoxified via detoxification enzymes (Schuler 1996, Simmonds 2003, Chen et al. 2015, Pan et al. 2016).
https://academic.oup.com/jinsectscience/article/20/1/8/5736528 (Yuan, YF et al 2020)

Narrowly:
“Tannins are naturally occurring water-soluble polyphenolic compounds capable of binding to proteins in solution (Haslain 1979; Swain 1979). Tannins have been accorded a major role in plant-herbivore interactions (Feeny 1976; Rhoades and Cates 1976)”
https://sci-hub.se/https://link.springer.com/article/10.1007/BF00380074 (Karowe, DN 1989)

Goal:

- A) Identify one demonstration pathway of a plant phytochemical through the food chain to bats.
- B) Then, (not in scope of the present work) generalization can proceed, as via:
    - 1) other relevant phytochemicals,
    - 2) plants producing them,
    - 3) other insect species, and
    - 4) other bat species.

Goal part A): Demonstration pathway:
Birch tree leaves -> tannic acid -> l. dispar (moth) -> m. schreibersii -> fecal sample PCR assayed -> inhibition of PCR polymerase

- the plant in the demonstration pathway is typically known for its herbivory by l.dispar
- tree foliage such as birch - tannic acid is very common and easily citable in tree foliage).

Goal A) Demonstration Pathway:

1. Isolate any one species of bat:

minopterus schreibersii
Common name: Schreiber's bent-winged bat

2. Identify one species of this bat's dietary insect prey:

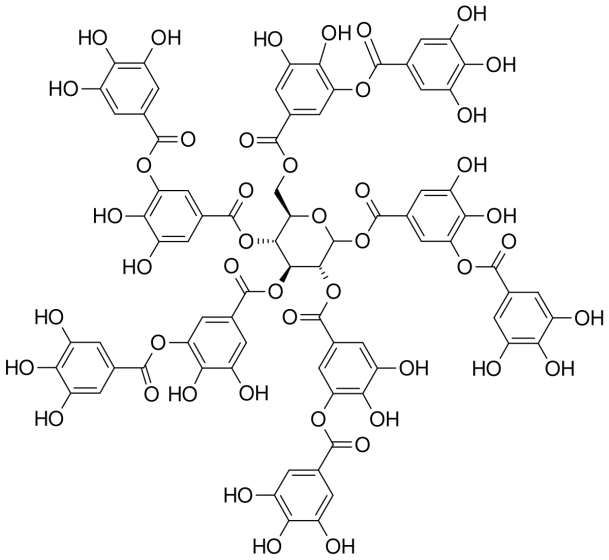
Lymantria dispar,
common name: gypsy moth)

Supporting citation 1:
"In June 2006, lactating [*m. schreibersii*] females benefited from an outbreak of Asian gypsy moth (**Lymantria dispar**) in holm-oak woodlands, and were probably attracted by this abundant resource" (Vincent et al. 2011)

Supporting citation 2:
"as well as the switch of foraging areas to deciduous woodlands, as a possible consequence of an outbreak of Asian gypsy moth (**Lymantria dispar**), during a radio-tracking study in southeastern France " -(Presetnik et al 2013 - "The diet of Schreiber's bent-winged bat, Miniopterus schreibersii (Chiroptera: Miniopteridae), in northeastern Slovenia (Central Europe)"

3. Identify one polyphenol that this insect consumes:

tannic acid



“When it comes to phytophagous insect species, plant secondary metabolites are very important factors in choosing host plants for feeding and oviposition (Harborne 2001). Tannic acid belongs to a group of natural phenols (tannins) synthesised by plants. **Tannins** are high-molecular-mass polyphenol secondary metabolites and they **represent about 2–20% of leaf dry mass** (Haslam 1979). The effect of tannins on insects depends on their chemical structure and host plant species (Kraus et al. 2003). Concentration and type of tannins vary among populations of the same plant species (Laitinen et al. 2000) during seasons (Laitinen et al. 2000; Covelo & Gallardo 2001) and stage of leaf development (Riipi et al. 2002). ***Lymantria dispar*** caterpillars feed on more than 500 different plant species during spring and summer (Lance 1983). Oak is a suitable host plant for them, and the level of **tannins** in oak leaves goes from very low in spring to high in autumn (2–4% fresh weight; Feeny 1968)”
Source: (Ilijin et al 2016)

B) Generalize pathway (only projected, not demonstrated at scale in present work)

[other insectivorous bats]
[other plants] -> [other bat species] -> [other moth & insect species] -> [other polyphenols] -> fecal sample PCR assayed -> inhibition of PCR polymerase

Examples of other polyphenols: 1. other tannins 2. Flavonoids

Discussion

Known mitigation of PCR inhibition in practice

The forensic analysis industry takes into account the presence of PCR inhibitors from samples (McCoord 2015)

Bovine serum albumin is a commonly proposed solution for PCR purposes - one would want to ensure it's implemented and implemented well. Hard to argue against the use of a control assay of a known amplicon in a partition from the same sample to make sure its working

WIV protocol does mandate the use of BSA in PCR at an ostensibly strong concentration (Chmura 2017) : So then one would hope that most other labs have same in their protocols. But the appropriate concentration remains a question: As there are many unrelated motivations to put BSA into an assay besides for neutralizing inhibitors, it is unknown if WIV's protocol was designed to follow industry trade practices for mitigating significant concentrations of PCR inhibitors .

Conclusion

The inhibition of PCR polymerase by polyphenolic compounds contained in fecal samples of insectivorous bats is anticipated. It is recommended that PCR assays of bat fecal samples be appropriately provisioned to mitigate their inhibitory potential.

Further reading - (non-core bibliography)

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“Effect of Tannic Acid on the Development and Resistance of the Gypsy Moth Lymantria dispar L. to Viral Infection” (Martemyanov et al 2006)
https://www.researchgate.net/publication/6808891_Effect_of_tannic_acid_on_the_development_and_resistance_of_the_Gypsy_moth_Lymantria_dispar_L_to_viral_infection

Insects interactions with flavonoids

https://www.researchgate.net/publication/12089683_Importance_of_flavonoids_in_insect-plant_interactions_Feeding_and_oviposition
“Importance of Flavonoids in Insect-plant interactions: Feeding and oviposition”
‘Jeffrey Harborne and colleagues have been responsible for collating the majority of data on the role of flavonoids in insect plant interactions.’

http://oar.icrisat.org/6082/1/AJAA_4_4_298-307_2012.pdf
“Effect of Some Flavonoids on Survival and Development of Helicoverpa armigera (Hübner) and Spodoptera litura (Fab) (Lepidoptera: Noctuidae)”

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6749194/
“Natural Products Diversity in Plant-Insect Interaction between Tithonia diversifolia (Asteraceae) and Chlosyne lacinia (Nymphalidae)”

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3925296/
“Are Naringenin and Quercetin Useful Chemicals in Pest-Management Strategies?”

insects sequester flavonoids:

https://www.researchgate.net/publication/10591625_Flavonoid-insect_interactions_Recent_advances_in_our_knowledge
“Flavonoid-insect interactions: Recent advances in our knowledge”

During long-term co-evolution with insects, plants developed a series of physical, chemical, and developmental defense mechanisms to prevent insect feeding, among which chemical defense systems involving secondary metabolites are the most important (Gong and Zhang 2014). To respond plant defense, insects developed multiple resistance mechanisms against plant secondary metabolites (Ivie et al. 1983, Zhu-Salzman and Zeng 2010). From the perspective of plants, strategies to prevent phytophagous insects from feeding on plants are beneficial, including antifeeding, repelling, and poisoning strategies as well as the inhibition of insect growth and development (Kelly and Curry 1991, Panzuto et al. 2002). From the

perspective of phytophagous insects, ingested plant secondary metabolites can be tolerated or detoxified via detoxification enzymes (Schuler 1996, Simmonds 2003, Chen et al. 2015, Pan et al. 2016).

From https://academic.oup.com/jinsectscience/article/20/1/8/5736528

The study of tannin-insect interactions has been hampered by poor communication between chemists and biologists.

https://link.springer.com/chapter/10.1007/978-1-4684-7511-1_26

Presence of the polyphenol in plants
H. Robles, in Encyclopedia of Toxicology (Third Edition), 2014
Tannic acid is a naturally occurring plant polyphenol and can be found in practically all aerial plant tissues. (Note from Rick - this is possibly an overstatement but you get the idea)

I. Dispar eats tree foliage (but not correlated specifically to tannic acid in this piece)
https://www.fs.usda.gov/treesearch/pubs/46007

(Another moth species:]
Larvae of the pomegranate fruit moth (Ectomyelois ceratoniae) develop on immature and mature pomegranate fruit which contain a considerable amount of tannic acid ranging from 85 to 168 ,ug/g fresh weight of tissue (Al-Izzi and Al-Maliky, 1986).
https://sci-hub.se/https://www.sciencedirect.com/science/article/abs/pii/S0022474X96000252?via%3Dihub
/ https://www.sciencedirect.com/science/article/abs/pii/S0022474X96000252

During the last two decades, a number of studies have built up, showing that the effect of host plant resistance is delivered through the food chain via herbivores to next participants of the chain (reviewed in [12]). This phenomenon which was proposed by several groups of re- searchers showed the direct effect of highly-reactive toxic plant molecules on entomopathogens (consumers of the second level) within the midgut of insects (consumer of the first level) [13– 14].